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Diffusion-Based Method and System For Detecting and Monitoring Activity of Biologic and Chemical Species

Cross Reference to Related Applications

This application claims the benefit of U.S. Provisional Application no. 60/375,668, filed April 26, 2002

Background

[0001] Biologic systems and complex chemical processes, such as biochemical pathways, cellular activities, synthetic organic processes, and molecular interactions, (collectively referred to as biologic or chemical activity herein) pose considerable challenges to scientists interested in directly monitoring activities. Such systems usually are rather complex, existing in environments where a number of differing activities are occurring simultaneously, and are thus noisy. Currently, there are a number of general techniques routinely used for detecting biochemical phenomena [David Freifelder, Physical Biochemistry, 1982, second edition, W. H. Freeman & Co., New York], most of which employ techniques in which one or more of the constituents of the system is labeled in some fashion; often times, these labeling approaches show whether or not a particular event has occurred, such as the binding of one molecule to another, or altered activity of a particular step in a biochemical pathway [D. E. Koshland, 1970, The Molecular Basis for Enzyme Regulation, in The Enzymes, P. Boyer, Ed., 341-396, Academic Press]. A very limited number of techniques utilize the measurement of properties which directly measure some physiologic property of a system, or do not require the attachment of a label. However, in these cases, only a very limited amount of information is available, and in most cases, the techniques are difficult to carry out,

and thus the throughput is extremely limited.

[0002] Many conventional systems designed to monitor biologic or chemical activity utilize a sample containment structure in which the volume of the buffer constituent is many times the volume of the biologic or chemical species of interest. . In such systems, the relatively high buffer volume tends to reduce the system's sensitivity of detecting and measuring the activity of the biologic or chemical species within the buffer.

[0003] Fig. 1A illustrates a conventional containment structure for detecting and monitoring biologic or chemical activity. A reservoir 110 (e.g., a microtiter well) contains a small population of cells 113 within a buffer solution 115, in volumes which are typical of currently available hardware for growing and studying cell populations. In this case, the reservoir 110 may contain, for example, a milliliter or so of a sodium chloride buffer, and at the bottom of the reservoir 110, a population of cells 113 grow, typically in the range of 50,000 cells or so in total. One milliliter is 10^{12} cubic microns, and for a typical cell of radius 20uM, a population of 50,000 cells gives a total volume of ~$4x10^{8}$ cubic microns. Thus, the ratio of volume in the cells to volume in the reservoir is $4X10^{8}:10^{12}$, so that the cells occupy about four-ten-thousandths (4/10,000) of the total volume.

[0004] Fig. 1B illustrates a common physiological event in the human nervous system: the transmission of electrical impulses along neurons via the modulation of sodium ion channels in the cell membrane. In this example, the usual resting state of a cell is to have characteristically low concentrations of sodium internal to the cell, but residing in a buffer with relatively high concentrations of sodium (typically ~150millimolar). When the neuron is excited, sodium ion channels on the cell membrane transiently open, and allow passage of the extra-cellular sodium across the membrane, and into the cytosolic fluid (the fluid inside the cell). For the typical volumes in the configuration described above, a large flux of sodium ions from the extra-cellular buffer to the intracellular region would result in conductive changes in the range of at most 0.01%, and probably much less. Thus, measuring the conductivity of the buffer, and changes thereof due to transport of sodium ions into the cell, may not yield an appreciative change as a result of ion flux, since the buffer is effectively an infinite

reservoir for sodium ions. In addition to the small overall change in the concentration of sodium chloride in the system, this change will be limited to a small volume around the cells (at least initially). This is because the time for diffusion to occur and a new equilibrium to be reached in such a relatively large system, is very long. Thus, fast transient events are very difficult to detect, due to diffusion time limitations, and even more difficult to model.

[0005] Accordingly, there is a need to provide an improved system and method for monitoring biologic and chemical activities in a highly parallel fashion and at low material quantities

Summary of Invention

[0006] The present invention now provides systems and methods for detecting and monitoring activity of cellular or molecular species at extremely low quantities using the diffusion processes of, or driven by, the biologic or chemical species. These methods and systems are preferably fabricated in micro-scale systems such as microfluidic and/or photolithograhphically fabricated devices, which can be measurably interrogated in highly parallel fashion.

[0007] In one embodiment of the invention, a diffusion-based method for detecting activity of a biologic or chemical species is presented. The method includes supplying the biologic or chemical species to a finite volume diffusion channel having a transport axis. The method further includes supplying a reactive constituent in fluid communication with the biologic or chemical species, whereby the reactive constituent is known or suspected of being reactive to the biologic or chemical species. The method further includes detecting the presence or absence of a diffusion gradient occurring between the biologic or chemical species and the reactive constituent. The presence or absence of the diffusion gradient can then be correlated to the presence or absence of activity of the biologic or chemical species.

[0008] Additional embodiments of the invention are described and shown in the following drawings and detailed description.

Brief Description of Drawings

[0009] Fig. 1A illustrates a conventional containment structure for detecting and

monitoring biologic or chemical activity.

[0010] Fig. 1B illustrates the transmission of electrical impulses along neurons via the modulation of sodium ion channels in the cell membrane.

[0011] Fig. 2 illustrates a first embodiment of the detection/monitoring system in accordance with the present invention.

[0012] Fig. 3 illustrates one embodiment of a finite volume diffusion channel in accordance with the present invention.

[0013] Fig. 4 illustrates a process for computing the net amount of biochemical species X between transported between two measurement points in accordance with one embodiment of the present invention.

[0014] Fig. 5 illustrates an exemplary Na^+ diffusion response as measured within the detection system of Fig. 2.

[0015] Figs. 6A-6D illustrate in succession an exemplary Na^+ diffusion response propagating along the diffusion channel of the detection system of Fig. 2.

[0016] Fig. 7 illustrates a process for characterizing fast events in a biologic or chemical system in accordance with one embodiment of the present invention.

[0017] Fig. 8 illustrates a second embodiment of the detection/monitoring system in accordance the present invention.

[0018] Fig. 9A illustrates a massively parallel detection/monitoring system in accordance with the present invention.

[0019] Fig. 9B illustrates one embodiment of a fluidic/measurement cell 950 shown in Fig. 9A.

[0020] Fig. 10A illustrates a diffusion channel in accordance with one embodiment of the present invention.

[0021] Fig. 10B illustrates a diffusion response of the diffusion channel shown in Fig. 10A.

[0022] Fig. 11A illustrates a funnel-shaped diffusion channel in accordance with one embodiment of the present invention.

[0023] Fig. 11B illustrates a diffusion response obtained using the diffusion channel geometry of Fig.11A in accordance with one embodiment of the present invention.

[0024] Fig. 12 illustrates a multiple bead and measurement stage diffusion channel in accordance with one embodiment of the present invention.

[0025] Fig. 13A illustrates a method for detecting activity of a bio/chemical species in accordance with one embodiment of the present invention.

[0026] Fig. 13B illustrates a method for monitoring activity of a bio/chemical species in accordance with one embodiment of the present invention.

[0027] Figure 14 illustrates a map for correlating changes in measured conductivity to cellular activity in accordance with the present invention.

Detailed Description

[0028] Table of Contents

[0038]

[0039] *I. Definitions*

[0040] As used herein, the terms "biologic or chemical species" or "bio/chemical species "refers to structure whose biologic or chemical activity is primarily sought. The bio/chemical species refers to any chemical or biologic structure, including, but not limited to: small molecules such as organic and inorganic chemical compounds, proteins and peptides, lipids, nucleic acids, polysaccharides, ionic species, cofactors, synthetic compounds of molecular weight less the 500D intended for therapeutic purposes, other endogenous structures such as hormones, signaling peptides, neurotransmitters; larger biologic structures such as proteins, lipids, polysaccharides, antigens and antibodies, protein receptors and signaling structures, nucleic acids, larger proteins and protein complexes such as ion channels, other membranous protein structures, cell membranes, intracellular structures such as endoplasmic reticulum and organelles, prokaryotic and eukaryotic cells, yeast and fungi, ordered and random populations of cells, tissues comprised of cells obtained from living and non-living organisms, and the like.

[0041] As used herein, the term "reactive constituent" refers to a biologic or chemical structure which is known, or suspected of being reactive with the bio/chemical species, defined above. For example, in an embodiment in which the bio/chemical species consists of a molecular structure, the reactive constituent may comprise a molecule which is known or suspect of having a binding affinity thereto. In an embodiment in which the bio/chemical species consists of a cellular structure, the reactive constituent may comprise a small molecule which binds to the cell, or another constituent which is metabolized or otherwise alters the cells function. Examples of the forgoing include, but are not limited to: ionic species, molecules within the cell which are involved with metabolic activities, such as nutrient catabolic or anabolic processes; intermediate molecular constituents in a biochemical pathway, including regulatory molecules and signaling molecules; signaling molecules, such as neurotransmitters and hormones, as well as other peptidic and non-peptidic organic compounds; small molecules intended for therapeutic purposes, such as drugs and other molecules known or suspected of being biochemically active in a given cell population, and the like.

[0042] As used herein, the term "source constituent" refers to the mobile biologic or chemical structure which diffuses toward a "sink constituent", the term "sink constituent" referring to a biologic or chemical structure which takes up, metabolizes, absorbs, or otherwise reacts with the source constituent. The bio/chemical species or the reactive constituent may each function as the source constituent in different embodiments under the present invention. For example in one embodiment, the bio/chemical species being studied consists of a small molecule (source constituent) which diffuses toward a protein receptor (the reactive constituent and sink constituent). In another embodiment, the bio/chemical species studied is a cell operating as a sink constituent, the cell taking up sodium ions from a supply of sodium chloride which functions as the source constituent and the reactive constituent. These are only a few of the exemplary embodiments within the scope of the present invention.

[0043] As used herein, the terms "finite volume diffusion channel" or "diffusion channel" refer to an enclosed or unenclosed structure configured to contain a fluid volume which ranges between 1/100,000 th to 2,000 times the aggregate displaced volume of the sink constituent. In general, the sensitivity in the measured diffusion rate will be inversely proportional to volumetric ratio of the diffusion channel/sink constituent. In particular embodiments, the diffusion channel will contain a volume within the following ranges expressed in terms of the aggregate displaced volume of the sink constituent: between 1/100,000 th to 1/10,000 th ; 1/10,000 th to 1/1,000 th ; 1/1,000 th to 1; 1 to 10; 10 to 100; 100 to 1000; and 1000 to 2000.

[0044] As used herein, the term "activity" refers to biologic, chemical, or biochemical activity of the aforementioned bio/chemical species, some non-exhaustive examples being the pairwise binding of two biologic or chemical species such as occur in the binding of a small molecule to a protein receptor; the binding or interaction of three or more biologic or chemical species, such as occur in the binding of protein complexes; more complex serial or parallel interactions of any number of biologic or chemical species, such as occur in biochemical pathways for the metabolic and signaling activities in biologic systems; chemical binding and reactions, such as enzymatic catalysis, oxidation-reduction reactions, chemical signaling though hormones or chemokines, and other types of chemical reactions; other types of

biologic activities, such as neuronal signaling via membranous ion channel opening and closing; anabolic activities such as protein synthesis and expression, nucleic acid synthesis and expression; assembly and fabrication of more complex biologic structures such as the cytoskeleton, membrane-resident protein association and transport, cellular and organelle replication; and morphologic activities, such as storage and transport of biologic or chemical species, protein expression and secretion, intercellular communications though diffusion or transport of biologic or chemical species, cellular motility and intercellular interactions, and the like.

[0045] As used herein, the term "diffusion" refers to the process by which the source constituent moves from one location to another, usually via some prescribed thermodynamic properties such as temperature, concentration, chemical potential and gradients therein, as well as geometric properties such as length and cross-sectional area.

[0046] In one embodiment, a difference in concentration of the source constituent is introduced between at least two points within the diffusion channel, and the diffusion of the source constituent between these two points is detected and/or measured. In another embodiment, a source constituent and a sink constituent are provided in fluid communication with the diffusion channel, and the diffusion of the source constituent within or through the diffusion channel is detected and/or monitored. The present invention is not limited to detecting/monitoring concentration gradients as a means to monitor activity of bio/chemical species. In a similar manner, gradients in other quantities such as temperature, pressure, pH, chemical potential, charge, viscosity, density, color, absorption or emission spectra, polarization, biochemical, radiometric quantities, enzymatic activity, nucleic acid hybridization, molecular binding rates or affinities, and the like may be alternatively used as a means to monitor activity of the bio/chemical species under study.

[0047] As used herein, the term "transport axis" refers to the axis of the finite volume diffusion channel along which the source constituent migrates or is transported. In one embodiment, the transport axis consists of the major axis of the finite volume diffusion channel. In another embodiment, the term "transport axis" notwithstanding its singular spelling, comprises two or more axes, along which the source constituent

diffuses.

[0048] As used herein, the term "electrical signal(s)"or electrical measurement(s)" refers to a time-varying (ac) or time-invariant (dc) signal, the former operating over at any useful and measurable frequency. In one embodiment, a time-varying electrical signal extends over the Low frequency (LF) to millimeter-wave frequency range (1 KHz-100 GHz). In another embodiment, the time varying electrical signal extends into the conventional optical spectrum, e.g., in the range of 7000 to 4000 Angstroms . Time varying electrical signals occurring at both higher and lower frequencies may be used in additional embodiments of this invention, including x-ray frequencies.

[0049] As used herein, the term "signal coupled" refers to the condition in which the described elements permit an electrical signal to be communicated therebetween. As used in the art of electrical/electronic patents most commonly, "signal coupling" may occur between directly contiguous structures, as well as non-contiguous structures having one or more intervening elements. Further as known in the art of electronics, "signal coupling" may occur either through the direct contact of the described elements, or between elements not in physical contact through electrostatic, magnetostatic, or electromagnetic coupling.

[0050] *II. Overview*

[0051] Advances in small scale fabrication techniques, both electronic (e.g., semiconductor processing, micro-electromechanical systems or MEMS, as well as soft lithography) and non-electronic (e.g. micro fluidics), now allow the manufacture of structures whose typical dimensions are roughly comparable to many structures of biologic interest, such as cells, cell membranes, organelles, micro-beads with various biologically relevant structures attached to them, and larger molecules. In addition, these fabrication techniques are highly reproducible, and can be used to construct geometries which are very regular and easily amenable to modeling and simulation of the behavior of systems contained therein.

[0052] The electronic embodiments of these structures are capable of addressing and conveying signals to very small spatial regions, as well as to create topologically complex circuits of very high density. Application of micro-fabrication techniques has

enabled the capture and management of very small fluid samples in microfluidic structures, in which both dissolved and non-dissolved biologic and chemical constituents may be manipulated. In such an environment, active transport and passive diffusion (collectively referred to as diffusion) of dissolved molecules and other structures can be detected, simulated, modeled, controlled, and monitored in a highly reproducible way. The transport of constituents in these systems, the way in which compounds move from one region to another--depends on the activities of active components of such systems, as well as the geometries of the systems. By creating well controlled and easily simulated environments for diffusion to occur, and monitoring these processes, one can obtain a depth of understanding about the behavior of a whole range of biologic, chemical, and molecular systems.

[0053] The aforementioned capabilities can be used to provide platforms in which complex biologic and chemical systems can be simulated, prepared, managed, detected, and monitored, all in a highly parallel manner. The result is a collective ability to carry out very complex experiments on biologic and chemical systems, monitor the progress and conditions of the experiment, and to measure useful data on a variety of experimental parameters.

[0054] *Diffusion Processes*

[0055] Diffusion refers to a process by which some quantity either matter or energy moves from one location to another, usually via some prescribed thermodynamic properties such as temperature and concentration, as well as geometric properties such as length and cross-sectional area. As is well-known in the art, the basic equations governing diffusion processes are:

[0056]

$$j(x,t) = -D\nabla f(x,t)$$

$$\nabla^2 f - \frac{1}{a^2}\frac{df}{dt} = 0$$

[0057]

Here, j is a vector describing the flux density of the quantity which is diffusing (such as heat or molecules), f is a function describing the physical property responsible for driving the diffusion process (such as gradients in temperature or

concentration of molecules), and D is the constant of proportionality, called the *diffusion coefficient*, or *diffusivity*. For cases in which the transport properties are desired to be modeled, the first of these equations may be used. For cases in which the explicit time dependence is required, the second of these equations is used.

[0058] To solve this equation practically, one needs to know boundary and initial conditions of the problem being modeled. The boundary conditions refer to the values of the function f at specific points or regions within the system being modeled. In many cases, one specifies a particular geometry (such as a rectangular box, or a cylinder), then proceeds to apply constraints on the values of f at particular parts of the specified geometry. Initial conditions refer to the state of the system at some pre-defined starting point. In many cases, one does this by specifying the value of f at every point in the system at some initial time t. With these parameters specified, one can solve the equation in a forward manner, to yield the behavior of the system at a future time, based on the behavior and properties at a previous time; or equivalently, one can solve for any given parameter in terms of the other parameters. Important special cases include the situation in which there is no time-dependence, the so-called steady state where the function f does not depend explicitly on time. In this case, one is usually interested in modeling the transport of some particular quantity in the system form one region to another. A second special case is where one is interested in modeling the approach to equilibrium. In this case, initial conditions are specified (along with diffusion constants and geometry), and the system is allowed to evolve until the steady state is reached.

[0059] Diffusive processes, such as heat flux, or the transport of chemical species down a concentration gradient, is driven by many factors, such as concentration, temperature, viscosity, characteristic dimensions and shapes of the system, as well as sources (elements which add a particular quantity to the system) and sinks (elements which subtract a particular quantity from the system). In the latter case, one has to modify the diffusion equation to include source and sink terms..

[0060] *Conductivity*

[0061] In several embodiments of the present invention, the presence and/or rate of diffusion, which indicates biologic or chemical activity, is measured through the use of

electrical signals. In such embodiments, the parameter of conductivity may be used to electrically sense the presence and rate of diffusion.

[0062] Electrical conductivity refers to the capability of a given material to carry an electrical current, either in the form of a direct current (DC), or an alternating current (AC). Conductivity is described by the following equations:

[0063]

$$j = \sigma E = -\sigma \nabla \Phi$$
$$\sigma = \sum_i n_i |q_i| \mu_i$$
$$\mu_i = \frac{v_i}{E_i}$$

[0064] The first of these equations is a form of the diffusion equation, in which the quantity being transported is electrical charge, and the driving function is the gradient of the electrical potential, or voltage. Here, j is the current density, E is the electric field, and σ is the conductivity (which is essentially the diffusion constant for this special case.) In the second equation, the conductivity is the sum over all charge carriers with respect to their concentrations n, absolute value of the net charge q, and mobility μ. The mobility is defined to be the average velocity of a charge or charge carrier, divided by the average electric field acting on the charge or charge carrier.

[0065] An extension of the above equations which pertain to the present invention is the case where there are bulk transport properties of a given system which are not steady state, i.e., they vary with time. Usually this time dependence is due to the fact that there are sources present, as well as means to dissipate particular quantities. A more complete description of these types of phenomena is given by the Boltzmann transport equation:

[0066]

$$\frac{\partial f}{\partial t} + v \bullet \nabla_x f + \dot{v} \bullet \nabla_v f = \frac{df}{dt}$$

[0067] Here, $f=f(x,v,t)$ is the classical distribution function (as a function of position x, velocity v, and time t), which gives the ensemble distribution of positions, velocities, and the time-dependence thereof. Solving this equation gives the time rate of change

for the physical properties of a system responsible for the previously described behavior of diffusion and conductivity, in these special cases.

[0068] While conductivity is used as an indication of concentration in several exemplary embodiments described herein, the reader will appreciate that other electrical parameters such as resistivity or resistance, voltage, current, phase, impedance, inductance, capacitance, permittivity, permeability and the like may also be used as an electrical measurement parameter to detect changes in concentration, and accordingly, the presence or rate of activity of the bio/chemical species under investigation.

[0069] *Chemical and Biochemical Processes and Pathways*

[0070] The term "biochemical process" refers to a variety of biological behavior at the chemical level, including, but not limited to: enzymatic catalysis of reactions involving metabolic reactants and products, the transport of specific chemical species from one region in the cell to another (such as across the cell membrane), signaling processes such as occur in hormonal responses, or the transport of neurotransmitters across the synaptic cleft causing the initiation of signal propagation along neurons.

[0071] The term "biochemical pathway" refers to two or more individual reactions in series, resulting in a sort of chain-reaction or cascade of biochemically-mediated events. Pathways in biologic systems often consist of a very long series of biochemical processes, with a host of regulatory components, branch points (where derivative pathways bifurcate), all functioning in a manner which may be highly non-linear.

[0072] A property of biochemical events is that they usually occur in a spatially distributed manner. For example, an increase of a particular constituent in a system at one place requires (for purposes of satisfying conservation laws) that the constituent be depleted at some other region. A good example is ion flux across a cell membrane: extra-cellular stores of a particular ion are depleted and intra-cellular stored are enhanced, in the event that there is a net flux of the ion into the cell. The behavior is made use of in the present invention to detection monitoring activity of biologic or chemical species as will be illustrated below.

[0073] *III. Embodiments of the Detection/Monitoring System*

[0074] The monitoring system of the present invention utilizes a diffusion channel of limited volume in which measurement signals of various modalities are used to monitor the presence and/or rate at which a diffusion gradient is established between at least two points along the diffusion channel. The particular measurement modality (electrical, photonic, radiometric, enzymatic, fluorescent, colorimetric, etc.) and correspondingly the parameter monitored (conductivity, optical intensity, rads, rate of substrate turnover in an enzymatic reaction, spectral properties) can be varied, the only requirement being that the measurement probe used is configured to support the propagation and/or receipt of the particular signal being implemented.

[0075] Diffusion occurs between a source constituent and a sink constituent which are in fluid communication with each other. The source constituent moves toward the sink constituent through the finite volume diffusion channel, producing a concentration gradient which can be detected and/or measured. Based upon the biologic or chemical structure of the sink constituent, the presence or absence of the source constituent's diffusion rate toward the sink constituent, and/or the rate of that diffusion, the activity of the source constitute can be ascertained. Analogously, knowledge of the biologic or chemical makeup of the source constituent and the aforementioned presence (or absence) and source constituent's rate of diffusion, conclusions as to the activity of the sink constituent can be made.

[0076] As defined above, the bio/chemical species whose activity is the subject of investigation may function as the source constituent or the sink constituent. In addition, the subject bio/chemical species may function as both a source constituent and a sink constituent during different phases of an experiment. As an example, during a first phase of an experiment a subject cell operating as a sink constituent is activated upon receiving a reactive constituent, the cell's activity detected and monitored by the diffusion of the reactive constituent through the diffusion channel. The cell, upon activation, emits a structure which becomes a source constituent during the second phase of the experiment, the source constituent diffusing toward and binding to or otherwise reacting with another structure (fixed or mobile) within the diffusion channel. Thus, in the first phase of the experiment the cell operates as a sink constituent, and in the second phase of the operation the cell operates as the

emitter of a source constituent.

[0077] The presence and degree of a diffusion gradient can be detected between the two or more measurement points using electrical, photonic, radiometric measurement system as well as other modalities (essentially, any measurement which established that something is moving form one region to another). Depending upon the characteristics of the diffusing quantity, one measurement modality may exhibit superior detection and measurement sensitivity over others. For instance, when attempting to measure a concentration gradient created through the movement of charged or ionized particles, the use of electrical signals may be preferable. Particularly, one can make use of the fact that many chemical species of biologic interest are either charged, or interact with charges, and thus will change the conductive properties of an aqueous sample in which they reside; and many physiologic constituents, such as cells and proteins, act as bio-processors to alter the composition of surrounding media. Thus, physiologic behavior, such as molecular interactions and metabolic cellular responses, either to intended stimulation or inhibition, or responses to stimuli that were not a priori known to have effects, are measurable through the effect they have on the extra-cellular medium in which they reside. Additionally, the response of cells or molecules can be directly monitored through the effect that a given sample volume has on the propagation of a signal.

[0078] In other embodiments in which optically opaque or fluorescing particles are diffusing through the diffusion channel, a photonic measurement apparatus will be preferred to determine concentration gradients. The reader will appreciate that the present invention is not limited to the use of any one measurement modality and that others may be used in alternative embodiments of the present invention. Further, properties other than concentration (e.g., temperature, pH, etc.) may be used alternatively, or in addition to detect and/or monitor diffusion.

[0079]

Fig. 2 illustrates a first embodiment of the detection/monitoring system 200 in accordance with the present invention. In this embodiment, the system 200 includes a source reservoir 220, a sink reservoir 230, and a diffusion channel 240 connected between the sink and source reservoirs 220 and 230. In a particular embodiment, the system 200 is constructed as a microfluidic structure using fabrication techniques

known in that art. In this embodiment, a passive transport system is used in which the source constituent 222 migrates toward the sink constituent 232 resident in the sink reservoir 230.

[0080] The source reservoir 220 is configured to contain a source constituent 222, which in this exemplary embodiment comprises sodium ions. The sink reservoir 230 is operable to contain the sink constituent 232, which also consists of sodium ions, the sink reservoir having a lower concentration thereof. Of course, other source and sink constituents may be used to detect activity in other bio/chemical species in alternative embodiments within the present invention.

[0081] The finite volume diffusion channel 240 operates as a gradient bridge between the source and sink reservoirs 220 and 230 and includes a transport axis 242, along which the source constituent 232 diffuses. In the illustrated embodiment, the diffusion channel 240 has one major axis (length) which defines the transport axis 242. In another embodiment, the diffusion channel 240 includes at least one additional major (or near major) axis (width and/or height) which permits diffusion therealong. Preferably in such embodiments, measurement probes are located to measure diffusion along these additional major axes.

[0082] The diffusion channel 240 further includes a plurality of (i.e., two or more) measurement probes 244 disposed along the transport axis 242. The measurement probes 244 are operable to detect the diffusion rate of the source constituent as it migrates along the transport axis 242. In the preferred embodiment, a change in the diffusing quantity's concentration is measured, although other properties such as temperature, pressure, pH, etc. may be used in alternative embodiments within the present invention. Optionally, one or more measurement probes 244 are positioned to measure concentrations within the source and/or sink reservoirs 220 and/or 230. The measurement probes 244 may be either in direct contact with the solution flowing along the transport axis 242, or the probes 244 may be designed to electromagnetically coupled a signal to/from the solution as it flows along the transport axis 242. In such cases, the measurement probe need not be in direct contact with the solution. As noted above, the present invention is not limited to electrical/electronic means of interrogation. Other measurement modalities such as

[redacted]

photonic, radiometric, or biochemical (e.g., in the form of immunoassays) may be used to detect and/or monitor the diffusion process occurring along the transport axis 242.

[0083] The sink and source constituents 222 and 232 may be one of any known components. For example, as shown in Fig. 2, the sink constituent 232 may contain the same component (sodium) as present in the source constituent 222, except in lower concentration. In this embodiment, the sink constituent may consist of cells which metabolize or otherwise consume the sodium, thereby causing sodium to diffuse toward the sink reservoir 230 along the transport axis 242. Consequently, a sodium diffusion gradient is set up along the transport axis 242, the presence of which can be detected and monitored using measurement probes 244. In one embodiment, the measurement probes use electrical signals (ac or dc) to measure electrical parameters, such as conductance, at two or more lateral positions. Differences in the measured conductance along the transport axis (or above a particular threshold level) will indicate that diffusion is occurring and that the cell population in the sink reservoir is actively taking up the Sodium. Other electrical parameters such as resistance, voltage, current, capacitance, inductance and the like may be used alternatively. Other measurement modalities, such as optical, radiometric, or biochemical measurement techniques may be used alternatively or in addition to interrogate the measurement probes to provide detection and monitoring information.

[0084] In a system such as is described in Fig. 2, the time evolution of the system favors a tendency toward equilibrium, in which the concentration of sodium in the sink reservoir 230 is equal to the concentration of sodium source reservoir 220. In the non-equilibrium state, there is a net transport of sodium down the transport axis 242, so that there is a net flow of sodium and chloride ions from source reservoir 220 to sink reservoir 230. The rate of this transport is given by solving the diffusion equation under the initial conditions of concentration as a function of position, and the geometry of the diffusion channel 240. The latter dependence is characterized by the cross-sectional area and length of the diffusion channel 240 connecting the source and sink reservoirs 220 and 230.

[0085] Because the conductivity of the solution is directly proportional to the number of charge carriers in the solution, the relative concentration of sodium and chloride ions can be determined directly from a measurement of the resistance across each of the measurement probes:

[0086]

$$R(t) = \int_{volume} \rho(x,t) d^3x$$
$$= \int_{volume} \frac{d^3x}{\sigma(x,t)}$$
$$= \int_{volume} \frac{d^3x}{n(x,t)|q|\mu}$$

[0087] In the simple case where the charge and mobility do not depend on position, and the concentration n is nearly constant for the small volume about each electrode, then the integral is trivial to solve, and the resistance becomes simply:

[0088]

$$R = \frac{l}{\sigma A}$$
$$= \frac{l}{n/q/\mu A}$$

[0089] Here, l is the length between the measurement probes, and A is the cross-sectional area. Thus:

[0090]

$$n(x,t) = \frac{l}{R(x,t)/q/\mu A}$$
$$= \frac{C}{R(x,t)}$$

[0091] For many practical applications, the constant of proportionality C is determined empirically, using solutions of known concentrations or conductivities.

[0092]

In the configuration shown in Fig. 2, the flux of sodium ions down the transport axis 242 can be monitored via monitoring the conductivity. In addition, the multiple measurement probes 244 enable one to determine the gradient of the concentration, by simply determining the concentration at each of the points of detection, and knowing the distance between them. From this data, and the use of the diffusion

equation already described, the net flux from one reservoir to another can be determined as a function of time.

[0093] Fig. 3 illustrates one embodiment of the finite volume diffusion channel 240 having length L in accordance with the present invention. The measured concentrations of the source (or sink) constituent at positions x=0 and x=L, (each at time t) represent the boundary conditions of the diffusion channel 240.

[0094] As depicted, the length L is much greater than the width or height. In this condition, the diffusion process becomes one-dimensional and can be modeled easily using the aforementioned diffusion equation. In an alternative embodiment, one of the other dimensions is enlarged to approach or equal the structure's length. In this embodiment, diffusion occurs across a two-dimensional plane, and the aforementioned diffusion equation can be solved in two dimensions, leading to a richer understanding of the biologic or chemical activity occurring.

[0095] The boundary conditions (measurement points) may be expressed in terms of a single function $f(x,t)$, where the values of this function are take at the endpoints of the diffusion channel, arbitrarily taken to be x=0 and x=+L (and thus x=0 is at the left terminus of the containment structure, as drawn above).

[0096] As a specific example, assume function f to represent the concentration of a particular bio/chemical species in the system, [X]. Further assume that the concentration of this quantity is held fixed at the boundaries, so that $f(x=0)=[X_1]$, and $f(x=+L)=[X_2]$. (The distance or position along the diffusion channel 240 is represented by the variable x) In this case, the concentration as a function of position along the diffusion channel 240 is given by the following linear relationship:

[0097]

$$f(x)=[X_1]+\frac{([X_2]-[X_1])x}{L}$$

[0098] Note that there is no time dependence in this expression, meaning that the system is in steady state as determined by the fixed boundary conditions. In this case, the gradient is a simple linear function across the diffusion channel 240, given by the following equation:

[0099]

$$\nabla f(x) = \frac{\partial f}{\partial x} = \frac{[X_2]-[X_1]}{L}$$

[0100] Given this knowledge (obtained for example by direct measurement of the concentrations), a known value for the diffusion constant (obtain, for example, by directly measuring diffusion rates is systems with known initial concentrations), and a known geometry of the system, one can then directly calculate the net transport of the bio/chemical species *X* through the diffusion channel 240. This quantity can be computed using the following equations:

[0101]

$$j(x,t) = -D\nabla f(x,t)$$
$$= -D\left[\frac{[X_2]-[X_1]}{L}\right]$$
$$Q_{net} = \int_A j \bullet dA$$
$$= -D\iint_A dydx\left[\frac{[X_2]-[X_1]}{L}\right]$$
$$= -DA\left[\frac{[X_2]-[X_1]}{L}\right]$$

[0102] The boundary conditions at the two measurement points, along with the regular geometry of the diffusion channel 240 between measurement points (here assumed to be a rectangular box with uniform cross-sectional area *A*) and the diffusion constant *D,* provides the current density *j* throughout the system. By integrating *j* across the cross-section of the conduit, the net transport *Q* of the bio/chemical species *X* between boundary points is determined. By extension, more complex geometries may be used, and time variation on the boundary condition can also be accounted for, using straight-forward extensions of the techniques used in this simple example.

[0103] Fig. 4 illustrates one embodiment of the aforementioned process for computing the total amount of bio/chemical species transported between two measurement points. Initially at 402, a diffusion channel is provided in which at least two measurement points z_1 and z_2 are provided, the measurement points z_1 and z_2 being separated by a dimension L. Next at 404, the change in relative concentration of the bio/chemical species X between measurement points z_1 and z_2 is determined, a

process by which can be performed using the systems and methods described herein. For example, the change in relative concentration of X between measurement points z_1 and z_2 can be obtained by using the described system to measure a change in the species" conductivity, and subsequently correlating that change to a change in concentration. As described above, different measurement modalities may also be used to detect/measure a change in the species" concentration.

[0104] Next at 406, the channel's cross-sectional area A between measurement points z_1 and z_2 is determined. This quantity may either be known from the fabrication specifications of the channel or measured (for example, though the use of a solution with known diffusion properties and concentrations, applied to the system described) if the dimensions are not known. In an alternative embodiment, either of the other three variables may be determined if the remaining variables are known.

[0105] At 408, the diffusion coefficient D of the particular bio/chemical species is determined. In the preferred embodiment this quantity is determined by measuring the diffusion rate of the bio/chemical species in an apparatus in which the geometry well-known and the concentrations are accurately measured. Such measurements may be made before, during, or after the process being described herein. For example, activities of interest (such as molecular binding or other biologic or chemical responses) as demonstrated in an experiment, may be subsequently studied via the explicit measurement of diffusion properties in a system designed for such study. In such a system, for example, the isolated diffusion properties may be evaluated by providing well-specified geometries of the fluidic system, and accurate concentrations of the biologic or chemical species, then measuring the diffusion rate of the species in the system. The result of such an experiment would be the determination of the diffusion constant, which would subsequently allow for the quantitation of the total transport of the species in the previous experiment. Of course, one could do the same procedure before an experiment as well.

[0106] At 410, the total amount of species transported is determined by calculating the quantity:

[0107]

$$DA\left[\frac{[X_2]-[X_1]}{L}\right]$$

[0108] Several observations can be made using the monitoring system of the present invention. First, the concentration gradient is a function of the difference in the two measured concentrations , as well as the linear distance L therebetween. Thus, to maximize the gradient, the distance L can be shortened (still assuming fixed boundary conditions), and/or the concentration difference at the two boundaries can be increased.

[0109] Secondly, the above diffusion equation can be accurately approximated by a one-dimensional model, with relatively simple boundary conditions. Using this model, one can then calculate the value for the difference in the concentrations between the boundaries, given a measured value for the gradient; or if the concentrations at one of the two endpoints is known (for example, by direct measurement, or by being prepared in some known way), then given the measured gradient, we can measure the absolute value of the quantity at the other boundary. In addition, these relationships can be used to calculate the total transport of material or energy from one region to another.

[0110] The above analysis assumes that the concentrations at the two measurement points remains fixed. However, in some instances these concentrations will vary with time. In these cases, there may be a complicated time dependence on the boundary conditions, which in turn will cause a more complicated form of the function f. This more elaborate representation of the function f can be used to provide richer information content on the systems being studied. Several cases of this are now presented.

[0111] *Case 1* : $f(x,t)$ at the boundaries is not a function of time.

[0112] This case reduces to the case previously described in Fig. 3, in which fixed boundary conditions result in a fixed gradient which is described wholly in terms of the geometry (length and cross-sectional area, in this example) and the concentration differences at the boundaries.

[0113] Analyses of such systems will reveal, for example, the rate of uptake of a

particular nutrient or metabolite of a given cellular system, if the nutrient or metabolite is added to the test reservoir. In this case, there may be a finite but large supply of the nutrient in the test reservoir, sufficient to hold the concentration in that reservoir nearly constant for the duration of the experiment; and the sample reservoir has some biologic system resident within its confines that is metabolizing the nutrient at a constant rate. The result is a time-independent set of boundary conditions, and thus a constant gradient across the gradient bridge. One can calculate directly the uptake of the nutrient or metabolite from a knowledge of the boundary conditions and the diffusion rate.

[0114] Note that systems like these can, among other purposes, serve as a calibration standard. For example, suppose one seeks to monitor the overall metabolic activity of a given set of samples, and use this as a reference by which other activities can be standardized. In this case, one simply places an excess of an essential nutrient in the test reservoir, excess meaning that there is far more of this nutrient than the sample population of cells can utilize in the course of the experiment and measures the gradient across the diffusion channel to determine the net flux of this nutrient from the reservoir to the cell population. As an extreme example, it is clear that a population of dead cells would not use any of the nutrient, and thus the gradient would be less steep (or even flat, at equilibrium) than a population of live, healthy cells.

[0115] Case 2: $f(x,t)$ at the boundaries is a slowly varying function of time.

[0116] In this case, "slowly varying" means that the concentration gradient in the diffusion channel has sufficient time to adjust to changes in the boundary conditions that it remains near equilibrium; the gradient is constant at any time during the experiment, but may change slope throughout the experiment. In this case, the problem may be reduced to solving the equilibrium situation, as described in case 1, for different values of the boundary conditions at different times (a so-called "piece-wise linear" situation).

[0117] In some embodiments in this case, the time variance behaves as a simple exponential function, whose time constant may be made arbitrarily large by the appropriate choices of the cross-sectional area A and the length L. Both of these

properties can be used to normalize data, as well as to separate simple diffusion-driven transport processes from true sources and sinks.

[0118] In addition, by varying the geometry of the diffusion channel, a filter for events on different time scales can be generated. For example, suppose the ion channel activities in the range of 100msec are of interest only, excluding the slower drift or diffusion events in the cell. A system can be constructed, by choosing the cross-sectional area and length such that the diffusion process being studied occurs on a timescale consistent with a desired temporal window of observation. Thus, with the appropriate geometries, slow events (such as simple diffusion out of the test reservoir and into the sample reservoir) yield piecewise-equilibrium type behavior, but faster events do not. For example, suppose the total time required for a particular ionic species to diffuse across the diffusion channel is one millisecond, and we are interested in studying the activity of a population of cells which have membrane ion channels specific for this particular ion which opens on a millisecond time scale. Consider also the case where this population of cells have, in addition to this fast mechanism of ion transport modulation, a secondary means for absorbing the ionic species which occurs over many seconds or minutes. In the case where the total diffusion time is on the order of a few milliseconds, these slow events will give rise to a shift in the overall gradient across the diffusion channel. However, since this process is sufficiently slow as to allow equilibrium to occur in the diffusion channel, the overall result is that the gradient remains constant (meaning that it is a linear function of the distance) within the diffusion channel. Contrast this to the result of sudden shifts in ionic concentration at one end of the diffusion channel owing, for example, to the opening of the fast ion channel under consideration which will result in non-equilibrium shifts in the concentration gradient across the bridge, and thus deviations from linearity in the gradient. These differences can be used to filter the responses of the gradient in the diffusion channel, and pick out the activities which are of interest to study.

[0119] *Case 3* : $f(x,t)$ at the boundaries is a rapidly varying function of time.

[0120] In this case, "rapidly varying" means that the concentrations and gradients therein do not have time to reach a new equilibrium on the time scale of the fluctuations in f

at the boundaries. In this case, continuing with the example above, the full spatial and temporal dependence of the concentration is preferably taken into account between measurement points along the diffusion channel. The general diffusion equation, which is solved for the time-dependence as well as the spatial dependence, is given by:

[0121]

$$\nabla^2 f - \frac{1}{a^2}\frac{\partial f}{\partial t} = 0$$

[0122] Fig. 5 illustrates an exemplary Na^+ diffusion response as measured within the detection system of Fig. 2. In this instance, a small population of cells is immersed in a standard buffer solution with ~150millimolar sodium chloride. At time $t=t_1$, the ion channels are stimulated, resulting in a transient drop and subsequent return to baseline at time $t=t_2$, in the concentration of sodium in the reservoir.

[0123] Now suppose that the interval between t_2 and t_1 is shorter than the diffusion time across the diffusion channel, the length of time it would take for a step function change at one of the boundaries to propagate across to the other side, and reach equilibrium. In this case, there would be a transient in the concentration that would propagate across the diffusion channel, as shown in succession in Figs 6A-D.

[0124] Thus, by profiling the gradient in the diffusion channel, and comparing that profile to computed or empirically-derived values for the diffusion properties of the system both the diffusion channel and boundary conditions, fast events in biological or chemical systems can be characterized, both with respect to the particular bio/chemical species involved, as well as to the temporal properties of such events. In this context, it is worth noting that for typical dimensions attainable in micro-fabricated structures, diffusion times down in the micro-second (10^{-6} Sec) range are achievable; thus very fast biological events can be monitored and studied.

[0125] Fig. 7 illustrates one embodiment of the aforementioned process for computing a diffusion response profile. Initially at 702, a diffusion channel is provided in which at least two measurement points z_1 and z_2 are provided, the measurement points z_1 and z_2 being separated by a dimension L. Next at 704, a first diffusion response profile is obtained, the first profile representing the change in relative concentration

of the bio/chemical species X between measurement points z_1 and z_2 is determined at time t_1. This process can be performed using the systems and methods described herein. For example, the change in relative concentration of X between measurement points z_1 and z_2 can be obtained by using the described system to measure a change in the concentration's conductivity, and subsequently correlating that change to a change in concentration. As described above, different measurement modalities may also be used to detect/measure a change in the species concentration.

[0126] Next at 706, a second diffusion response profile is obtained, the first profile representing the change in relative concentration of the bio/chemical species X between measurement points z1 and z2 is determined at time t2. Next at 708, a complete diffusion profile is assembled by combining the first and second diffusion profiles. Of course. additional diffusion profiles in which the differential concentration change is made at times t_3 -t_n may be made and combined to provide the complete diffusion response. The combined diffusion response can be stored and retrieved to compare to a later diffusion process in order to identify it.

[0127] Fig. 8 illustrates a second embodiment of the detection/monitoring system 800 in accordance the present invention. The system 800 includes a voltage source 802, resistors 804, and a voltmeter 806 in addition to the previously described diffusion channel 240 and measurement probes 244a and 244b. In the specific embodiment, probes 244a and 244b each include a high side probe ($244a_1$ and $244b_1$) and a low side/ground probe ($244a_2$ and $244b_2$). In contrast to the system 200 of Fig. 2 where the sink constituent resides outside of the diffusion channel 240, the sink constituent in the instant system 800 is deposited within an interaction region 820, preferably located between measurement probes 244a and 244b. The invention is not limited to the use of two-member probes, and "single" probe conductors such as wire, optical fiber, or waveguides, may be used in alternative embodiments within the present invention. Further alternatively, the voltage source 802 and resistors 804 are removed and voltmeter 806 is replaced by an ohm meter to passively measure the differential resistance occurring between measurement probes 244a and 244b.

[0128] The monitoring/detection system 800 is designed to detect/monitor the diffusion rate through sensing a difference in the measured conductance of probes 244a and

244b. Initially, a first voltage 802 to each high side probe member 244a $_1$ and 244b $_1$ through resistors 804 (source constituent flow is assumed left to right). In one embodiment of the invention, the probes and resistors are photolithograhphically formed, and accordingly exhibit little or no difference in resistance.

[0129] As the source constituent interacts with the sink constituent resident within the interaction region 820, the source constituent undergoes a change in concentration. The first and second measurement probes 244a and 244b will measure the source constituent's concentrations before and after the sink constituent, respectively, a difference indicating that a reaction, e.g., the sink's uptake of the source, has occurred.

[0130] The probe having the comparatively highest local conductance between its high and low side elements will sink the larger amount of current compared to the probe having a lower conductance . Accordingly, a voltage difference between the two probes will be developed, the magnitude and direction of the voltage difference (monitored by voltmeter 806) being indicative of the magnitude and direction of the diffusion process occurring. Of course additional measurement probes 244 may be positioned along the transport axis to detect or monitor smaller changes in the diffusion rate. In a particular embodiment, the fluidics and signal measurement systems may be integrated together on the same platform.

[0131] A similar technique can be used for other measurement modalities as well. For instance, in an ac-based measurement system, the diffusion rate may be detected or monitored by applying and monitoring a time-varying voltage taken at each of the probe positions 244. In such an embodiment, probes 244 would consist of connectors (or waveguides, depending the operating frequency) operable to support the propagation of the desired ac signal (low side measurement probes 244a $_2$ and 244b $_2$ would be connected to an ac ground, which may not be dc ground). Voltage source 802 would consist of an ac signal source operating at the desired frequency and amplitude, resistors 804 would be resistors or attenuators (depending upon the operating frequency), and voltmeter 806 would consist of an ac voltmeter. Further alternatively, the voltage source 802 and resistors 804 are removed deleted and voltmeter 806 is replaced by an ohm meter to passively measure the differential

resistance occurring between measurement probes 244a and 244b. Electrical parameters other than conductance and resistance (e.g., voltage, current, inductance, capacitance, etc.) may be used in alternative embodiments within the present invention.

[0132] The aforementioned measurement system could be fabricated as part of a micro-miniature, massive parallel system using know processes such as hybrid integrated circuit processes, monolithic integrated circuit processes, micro-electromechanical systems (MEMS). The fluidics of such a system may consists of a microfluidic system which is combined (e.g., monolithically integrated, or separately fabricated and attached) with the measurement system.

[0133] The aforementioned system may also be implemented in an optically-based measurement system. In such a system, the diffusion rate of the source constituent which contains, or is itself optically opaque may be detected by monitoring the optical intensity of a reflected signal at each of the probe positions 244a and 244b, a lower intensity (or different wavelength) being indicative of a higher concentration at that particular measurement probe. In this embodiment, measurement probes 244 are fiber pigtails or other optically transparent probes configured to support the propagation of an optical signal at the desired wavelength. The volt source 802 would be an optical source, resistors 804 would be optical attenuators of substantially the same value, and voltmeter 806 would consist of an optical intensity meter. Further alternatively, in systems using a fluorescing source constituent, the aforementioned optical source and attenuators may be deleted and the optical intensity meter may be replaced with an optical wavelength meter to measure the difference in wavelength emitted at the two measurement probes. Optical parameters other than intensity and wavelength (e.g., solution transparency) may be used to detect/monitor the concentration of the source constituent in alternative embodiments within the present invention.

[0134]

This measurement portion of the system could be fabricated as part of a micro-miniature, massively parallel system using fabrication techniques such as optical micro-electromechanical system (MEMS), or similar approaches. As disclosed above, other measurement modalities such as radiologic, biochemical, and others may be

used in alternative embodiments within the present invention. Preferably, the fluidics of such a system consists of a microfluidic system which is combined (e.g., monolithically integrated, or separately fabricated and attached) with the measurement system. Microfluidic systems such as those formed from the multi-layer soft photolithography process employed by the Fluidigm Corporation of South San Francisco, California, etched glass processes as employed by Caliper Technologies, Inc. of Mountain View California, and polymer plastic processes as employed by Aclara Biosciences, Inc. of Mountain View, California may be used in embodiments of the present invention.

[0135] Fig. 9A illustrates a massively parallel detection/monitoring system 900 in accordance with the present invention. As shown, the detection/monitoring system 900 includes N vertical fluid channels and M horizontal channels, each channel intersection forming a fluidic/measurement cell 950. The system 900 may consist of any parallel-arranged combined fluidic/measurement system, not excluding the aforementioned microfluidic, hybrid, monolithic, or MEMS systems, described herein.

[0136] Fig. 9B illustrates one embodiment of a fluidic/measurement cell 950 in accordance with the present invention. As shown, each cell 950 includes a horizontal flow channel 952, a vertical flow channel 954, and a cavity 956 operable to capture beads 959 introduced into the cell through vertical channel 954. Beads 959 operate as the sink constituent and contain either the bio/chemical species under study or the reactive constituent which is known or suspected of being reactive to the bio/chemical species under study. The bio/chemical species or reactive constituent are deposited on beads using conventional means know in the art of biology and chemistry, and the beads 959 may be composed of paramagnetic or non-ferrous material as known in that art.

[0137]

The horizontal axis of cell 950 defines the transport axis, along which measurement probes 244a and 244 b are disposed. The source constituent is provided through the input 952a, across the first measurement probe 244a, across one or more of the beads, across the second measurement probe 244b and to the channel output 952b. Although not shown, measurement probes 244 are connected to resistors, a voltmeter, and a voltage source as shown in Fig. 8. Preferably, each cell

is singularly addressable to provide a voltage signal thereto and to receive a voltmeter reading therefrom.

[0138] Initially, the beads 959 are supplied to cell 950 via vertical channels 954, one or more beads 959 collecting in the cavity 956. Next, the source constituent is supplied to the horizontal channel input 952a. The source constituent has a baseline, i.e. "unaffected" concentration as it passes across the first measurement probe 244a. As the source constituent passes around the one or more beads 959, its concentration may change if it reacts with the material (bio/chemical species or reactive constituent) attached to the surface of the bead 959. The solution passes across the second measurement probe 244b having a different concentration, the difference in concentration present between the first and second probes 244a and 244b being sensed and indicating the presence (or absence) and/or rate of activity in the bio/chemical species.

[0139] In the preferred embodiment, the vertical fluidic channel 954 connects to each of the M vertically oriented cells, allowing beads 959 to be supplied to each of those cells. Once each of the M cells capture one or more of the beads (perhaps as indicated by a predefined period of time elapsing), a parallel measurement process may be performed in which M different source constituents may be supplied to each M input. Subsequently, each cell may be addressed and interrogated to determine whether, and/or to what degree an interaction between the bead material and the particular source constituent is occurring.

[0140] Similarly, each of the horizontal fluidic channels 952 connects to each of the N horizontally oriented cells, allowing the supplied source constituent to travel through each of these cells. Preferably, each of the N vertically oriented fluidic channels 954 are supplied with beads having different attached material. Accordingly, activity of each of the M source constituent can be analyzed with respect to N different bead-based materials as each source constituent horizontally flows through each of the N cells. As described above, each of the cells may be individually addressed and interrogated in order to detect/monitor activity within them.

[0141] The finite volume diffusion channel of the present invention may be constructed of various cross-sectional areas in order to provide further detection and/or

monitoring benefits. Figs. 10-12 illustrate some of these embodiments.

[0142] Fig. 10A illustrates a portion of the diffusion channel 240 located between measurement probes 1030 (not shown), the diffusion channel portion having beads 959 and a bead flow restrictor 1010 in accordance with one embodiment of the present invention. The number of beads 950, flow rate, and dimensions of the diffusion channel can be selected such that desired diffusion response is obtained. Specifically, the source constituent flow rate, the length of the bead region 1020, and distance to the measurement region 1030, along with the total quantity of the target molecule within the bead region 1020 binding region and relative affinities, will determine when saturation occurs, and thus the binding kinetics as discussed below.

[0143] The bead flow restrictors 1010 may be any permanent or temporarily means to confined/restrict the flow of bead therethrough, including the application of a magnetic field to temporarily restrict the flow of paramagnetic beads. Flow restriction may be accomplished through electrophoretic or dielectrophoretic separation techniques, as are well-known in the art. Chemical preparation of the bead surfaces may include any of the well-known techniques (such as Au-thiol chemistries, or silane chemistries) for derivatizing surfaces with specific linker and attachment chemistries, for the purposes of attracting and retaining molecules, molecular structures and complexes, as well as cellular extracts, cells and tissues.

[0144] Fig. 10B illustrates a first diffusion response of the diffusion channel 240 shown in Fig. 10A. This response indicates the difference of Q when the solution passes through the bead region 1020, as a function of time. In the absence of any interaction or binding, or for any equilibrium process, this difference is necessarily zero.

[0145] Fig. 11A illustrates a funnel-shaped diffusion channel 240 having beads 950 and a bead flow restrictor 1010 in accordance with one embodiment of the present invention. This embodiment facilitates the measurement of a fast diffusion response occurring within the interaction region 820.

[0146] Initially it is assumed that only a relatively small amount of the source constituent is removed during passage through the interaction region 820, on a volume-per-volume basis. In this case, by making the volume of the interaction region 820

relatively large compared to the downstream diffusion channel 240 where the measurement probe 244b is located, a larger total amount of source constituent is removed from the solution as it passes through the interaction region 820. In the absence of any constriction in the flow path, this change in the quantity Q representing the source constituent will be transient, comprised of a very short distance along the longitudinal direction, and with a relatively short time required for diffusive processes to smooth it out and make it undetectable. By constricting the width of the diffusion channel 240, it is extended in length (by an amount roughly equal to the inverse fraction of the diminution of width), so that diffusion to equilibrium takes much longer (due to the increased diffusion length), and the spatial extent of the solution with altered values of Q is extended, facilitating the measurement process.

[0147] Fig. 11B illustrates an optimal diffusion response obtained using the diffusion channel geometry of Fig.11A. As shown, the response includes a falling edge 1132 and a rising edge 1134 which are within the time scale of the graph. Of course, other geometries may be used to capture the particular diffusion response sought. In this manner, unwanted diffusion responses which are either faster (having a relative fast fall and rise time) or slower (having a longer fall and rise times), may be filtered out from the detection/monitoring process by means designing the physical dimensions of the diffusion channel accordingly.

[0148] Fig. 12 illustrates a multiple bead and measurement stage diffusion channel 1240 in accordance with one embodiment of the present invention. The diffusion channel 1240 includes multiple stages of interaction regions 820 consisting of beads 959 and bead blockers 1010, each region 820 followed by a measurement probe 244. This configuration is useful for making serial analyses of a single source constituent with multiple sink constituents.

[0149] *Fabrication of the Detection/Monitoring System*

[0150] The detection/monitoring system of the present invention includes a fluidic system and measurement system which may be integrally formed or separately fabricated and attached. In the preferred embodiment, the fluidic system includes all of the structural and mechanistic apparati operable to isolate and transport samples

of fluid, along with non-fluid constituents residing therein, in the invention described herein. This structure may be fabricated using any of the well-known techniques in the art. Examples include, but are not limited to: hard or soft lithographic methods employing single or multiple layer materials (glass, plastics, polymers such as PDMS, synthetic and natural rubber, and other terpene-based matrices); mechanical methods of fabrication, such as micromachining of rigid and supple materials, using for example well-known micro-electrical mechanical systems (MEMS); the use of capillaries and other such conduits well-known in the art, as well as arrays of such structures when used in a multiplex fashion. Structures designed for fluid containment and transport may also be imbedded in larger systems, such as microtiter plates and wells, as are commonly used in wet lab applications.

[0151] The measurement system preferably includes all of the apparati operable to detect/monitor a signal originating from the interaction of the source and sink constituents. In some embodiments, the measurement system additionally includes apparati (such as signal sources and connecting circuitry) to provide an incident signal to the source constituent flowing through the diffusion channel, and in other embodiments the measurement process is passive and does not require transmitting a signal to the measurement probes. As discussed herein, the measurement system may be fabricated using MEMS techniques, semiconductor or photolithographic processing techniques, or other fabrication techniques known in the measurement modalities disclosed herein.

[0152] *IV. Detection and Monitoring Methodologies*

[0153] Figs. 13A and 13B illustrate, respectively, methods for detecting and monitoring activity of a bio/chemical species in accordance with one embodiment of the present invention. The activity detected includes biologic, chemical, or biochemical activity as defined herein.

[0154] Each method includes several processes, the sequence of which may vary in alternate embodiments within the present invention. Referring now to Fig. 13A, at 1302 the subject bio/chemical species under study is supplied in fluid communication with a finite volume diffusion channel as described herein. In this arrangement, the bio/chemical species may be located within the diffusion channel, or outside of the

channel within a reservoir that is fluidly connected to the diffusion channel. In the latter case, one or more fluid conduits constructed in a microfluidic or other micro-miniature structure referred to herein may be used. Next at 1304, a reactive constituent, which is known or suspect of being reactive with the subject bio/chemical species, is supplied in fluid communication with the finite volume diffusion channel. As discussed herein, either the subject bio/chemical species or the reactive constituent may operate as the mobile source constituent.

[0155] Next at 1306, a diffusion response occurring between the bio/chemical species and the reactive constituent is detected along the transport axis of the diffusion channel. This process is preferably accomplished by detecting a differential source constituent concentration occurring between two or more measurement probe positions, as described above. The diffusion response may be made at set time t_1, over a continuous time period $t_1 - t_2$, or at discrete times $t_1, t_2, \ldots t_n$. Lastly at 1308, the detected diffusion response is correlated to the presence or absence of activity of the bio/chemical species. In a particular embodiment of process 1308, the diffusion response detected in 1306 is compared against a baseline diffusion response in which the source constituent normally diffuses in the absence of the sink constituent. For example, the baseline response may be obtained using the same fluidic and detection system, but which lacks a sink constituent. The baseline diffusion processes in this structure can be detected and stored, for later comparison with one or more experiments which include a sink constituent. The difference in the measured diffusion properties reflects the effects of the sink constituent. In a similar manner, the measured and baseline responses may be obtained substantially concurrently using a parallel measurement system, such as that described in Figs. 9A and 9B above.

[0156] Fig. 13B illustrates a method the monitoring of activity of a bio/chemical species in accordance with one embodiment of the present invention. Initially at 1322, the subject bio/chemical species under study is supplied to a finite volume diffusion channel as described herein. As described above, this process may be performed by the use of fluid conduits constructed in a microfluidic or other micro-miniature structure referred to herein. Next at 1324, a reactive constituent, which is known or suspect of being reactive with the subject bio/chemical species, is supplied to the

finite volume diffusion channel.

[0157] Next at 1326, a diffusion response occurring between the bio/chemical species and the reactive constituent is measured along the transport axis of the diffusion channel. This process is preferably accomplished by measuring the differential source constituent concentration occurring between the two or more measurement probe positions, as described above. The diffusion response may be made at a set time t_1, over a continuous time period $t_1 - t_2$, or at discrete times $t_1, t_2, \ldots t_n$. Lastly at 1328, the measured diffusion response is correlated to activity in the bio/chemical species. In a particular embodiment of 1328, the diffusion rate measured in 1326 is compared to a baseline source constituent diffusion rate, in which it is know that a particular activity of the subject bio/chemical species is not occurring. The systems and processes discussed with regard to Fig. 13A by which a baseline response is obtained, either at a later time or concurrently with the measured response may also be used.

[0158] *Calibrating to Quantitate*

[0159] The method of Fig. 4 may be used to determine other quantities of the diffusion equation as described herein. As an example, the cross-sectional area A of the diffusion channel (of length L) may be solved for using the equation of Fig. 4 if all of the other variables are known. This could be accomplished, for instance, by measuring the diffusion of a known solution having a well-defined diffusion coefficient D, and computing the total amount transported from the measurement interval. Alternatively, the diffusion coefficient D may be computed if the other variables are known or can solved for independently.

[0160] *V. Examples*

[0161] *Example 1: Diffusion Channel Comprising a Reaction Vessel*

[0162] Initially, a small reaction vessel is provided. The reaction vessel meets the previously-described criteria of a finite volume diffusion channel, i.e., the volume of the reaction vessel is not greater than 10,000 times the aggregate displaced volume of the source constituent to be supplied to the vessel. The reaction vessel also includes two or more measurement probes configured to detect/measure the

diffusion of the source constituent, through the measurement of electrical means, such as conductivity.

[0163] Next, a cell population having a known, particular pathway is selected for study. As an example, cell populations having particular sodium ion channel behavior is selected. Subsequently, a viable and active population of the desired cells is established within the vessel, perhaps using buffers which are known to support the viability and functionality of the cell population. The conductivity of the system (cells + buffer) is then measured before stimulation, thereby establishing a baseline value.

[0164] Next, the cell population is stimulated by replacing the existing buffer with an identical buffer with a reactive constituent known to stimulate the pathway. Preferably, changes in the system's conductivity is monitored while adding the new buffer and a predefined time thereafter. A change in the monitored conductivity values can then be correlated to the cell's activity.

[0165] Figure 14 illustrates a map for correlating changes in measured conductivity to cellular activity. Four experiments are made: a first experiment 1401 in which sodium is added to the vessel and the cell's reaction is observed using an other measurement means (e.g.,, fluorescence); a second experiment 1402 in which the same amount of sodium is added and no change in activity is monitored; a third experiment in which no sodium is added and a change is cellular activity is monitored 1403; and a fourth experiment 1404 in which no sodium is added and no change in cellular activity is observed. The results of these experiments can be arranged in a matrix form, the first experiment indicating a positive response of the added sodium. Accordingly, the measured changes in conductivity can then be correlated to the cell's observed activity in later experiments..

[0166] *Example 2: Binding Kinetics*

[0167] A system such as that shown in Fig. 10A in which a population of beads is retained within a bead region, each of the beads coated with a specific protein to which small molecules are known or suspected to bin. The beads are isolated to a specific region of the diffusion channel such that the source constituent may emerge downstream. The diffusion channel includes measurement probes situated before and

after the bead region.

[0168] Next a solution containing the source constituent, a species of molecules or molecular structures which bind target proteins immobilized to the beads. When a solution containing the source constituent is introduced to diffusion channel, it will contain a certain concentration of the binding molecules. This concentration is measured before the interaction with the bead-immobilized target, then allowed to pass through the bead region at some determinable rate, and analyzed in the outflow section for the concentration of the molecular species of interest. The result of this measurement, combined with a knowledge of relative concentrations of target and molecule (or total amount of each) as well as the speed at which the solution moves through the bead region (or binding region), will yield the kinetic properties of the system directly.

[0169] The flow rate, and the length of the bead region, along with the total quantity of the target molecule in the bead region and relative affinities, will determine when saturation occurs, and thus the binding kinetics. In one case, where a relatively fast rate of flow is used, the diffusion response (an embodiment of which is illustrated in Fig. 10B) exhibits a sharp decrease in concentration where the initial quantity of X is removed from solution, a plateau region where the source constituent is being consumed, and a gradual return to the equilibrium value which of necessity is the same value as the solution in the in-flow region, since there is no net loss of the source constituent in the diffusion channel after saturation has occurred. In the case in which source constituent concentration is the same but its flow through the bead region is slowed, the response exhibits a much more step return to equilibrium as saturation occurs more quickly over this time period.

[0170] The bead surface represents the sink constituent in one embodiment of the present invention and may include any class of molecular structures, either bound to beads or other surfaces in the binding region, including derivatized and/or functionalized surfaces, polymer matrices or scaffolds, or molecules which reside in membranes such as are found in cellular systems or artificial membranes designed to mimic physiologic membranes; complexes of molecular structures, such as multi-protein complexes, or polymer-attached biomolecules of interest; or cell and/or

tissue samples which contain one or more molecular targets of interest, which may bind other molecular structures in the solution; and the like..

[0171] *VI. Applications*

[0172] The data obtained from such measurements may be applied in a variety of ways, and used for various purposes. For example, using data to generate gradient and transport properties of source constituents in the experiment which are known or are suspected, of being active, may result in an understanding of biologic activity or activities of the source or sink constituents. In the case of binding of a particular small molecule to a biologic target, as described above, the point in the system where this occurs will result in a local gradient. Other examples of analyses and uses include, but are not limited to: the transport of metabolically active molecules to specific cells or cell populations, which also will create local gradients, and indicate highly specific types of biologic or biochemical activities; the transport of signaling molecules, such as proteins and peptides, neurotransmitters and hormones, between cells or cell populations, resulting in local gradient changes whose measurement, along with the knowledge of initial conditions of the system will yield knowledge on activity or activities of the molecules; the use of gradients and other measurable changes in system properties (such as conductance or conductivity) as a means to monitor processes in the system, such as flow or tracking of samples within the system, and in addition, the use of this information to supply feedback data to system controllers (such as pumps and measurement devices, and components of the system designed to coordinate these functions).

[0173] While the above is a detail description of the present invention, it is only exemplary and various modifications, alterations and equivalents may be employed in various apparati and processes described herein. Accordingly, the scope of the present invention is hereby defined by the metes and bounds of the following claims: